

**BioPure™ MTAD™**  
**Antibacterial Root Canal Cleanser**  
**Research**

**ANTIBACTERIAL**

1. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. J Endodon 2003; 29(6):400-3.

- **Summary:**

- The purpose of this investigation was to test the ability of a mixture of a tetracycline isomer, an acid and detergent (MTAD) to kill *Enterococcus faecalis* and compare its efficacy to that of NaOCl and ethylene diamine tetraacetic acid (EDTA). The zones of inhibition and minimum inhibitory concentrations were measured for these solutions.
- Conclusion- Although EDTA demonstrated no antibacterial effects against *E. faecalis*, NaOCl and MTAD proved to be antibacterial to varying degrees. NaOCl continued to exert its efficacy up to 32x dilutions. On the other hand, MTAD was effective in killing *E. faecalis* up to 200x dilutions. Furthermore, although a 2 and 5 minute exposure of *E. faecalis* to undiluted or 1:2 dilution of NaOCl did not result in total killing of bacteria, exposure of the same bacterial species to undiluted or 1:2 diluted MTAD resulted in a completely negative culture after 2 and 5 minutes of exposure.
- Based on the results of this and previous investigations, it seems that MTAD has the ability to remove most of the smear layer and it possesses superior bacterial activity compared with NaOCl or EDTA when tested against *E. faecalis*.
- These results are significant because they demonstrate the efficacy of an irrigant to remove most of the smear layer and kill a bacterial strain that has been shown to be resistant to many commonly used intracanal irrigants and dressings.

2. Shabahang S, Manouchehr P, Torabinejad M. *In Vitro* antimicrobial efficacy of MTAD and NaOCl. J Endodon 2003; 29(7):450-2.

- **Summary:**

- The purpose of this study was to compare the ability of MTAD with that of NaOCl to disinfect human root canals that had been contaminated with whole saliva.
- Conclusion: The results of the investigation confirmed previous findings demonstrating almost 40% of the root canals remain infected using NaOCl and the superior antimicrobial activity of MTAD when compared with 5.25% NaOCl. An important finding in the study was the ability of MTAD to exert its antimicrobial effect during a brief time. This property is desirable in clinical practice where the root canal irrigants may be in contact with certain areas of the RCS for a short

time. Furthermore, this disinfecting property obviates the need for placement of an intracanal dressing necessitating multiple visits.

3. Shabahang S, Torabinejad, M. Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. J Endodon 2003; 29(9):576-9.

- **Summary:**

- The purpose of this investigation was to compare the antimicrobial effect of MTAD (a mixture of tetracycline isomer, an acid, and a detergent) with that of NaOCl (with and without EDTA) as a final irrigant on *E. faecalis*.
- Conclusion: The results from these samples in the positive control showed that, debridement of root canals using distilled water, is unable to sterilize the root canal system.
- The five samples in the negative control groups, which were exposed to the identical microbiological steps as the teeth in the experimental groups, demonstrated that the contamination did not occur during the culturing procedures. Absence of growth on the BHI plates using dentin shavings from this group confirmed that contamination did not occur during the preparation of the shavings.
- The ineffectiveness of NaOCl to consistently disinfect root canals in this study is in agreement with results from previous investigations. This finding is especially true with *E. faecalis*, which is a bacterium that is known to resist the antibacterial effects of certain root canal medicaments.
- Although the presence of the smear layer prevents penetration of antibacterial agents into the dentinal tubules, study results showed that removal of the smear layer with a one –minute rinse of 17% EDTA did not enhance the antimicrobial effects of NaOCl. The lack of increased antimicrobial efficacy may be due to the inability of NaOCl to remove the smear layer, its inability to penetrate into the dentinal tubules once the smear layer has been removed, or its ineffectiveness at killing *E. faecalis*. Findings also showed that removal of smear layer with a chelating solution (EDTA) that has little or no antibacterial effect, does not enhance the antibacterial effect of NaOCl.
- Fisher's exact test showed that the combination of 1.3% NaOCl as a root canal irrigant and MTAD as a final rinse was significantly more effective than the other regimens. The  $\chi^2$  test showed no difference between other regimens.
- On the basis of these results, it appears that MTAD is an effective final rinse for eradication of *E. faecalis* from extracted human teeth.

4. University of Helsinki, Finland: *E. faecalis* culture results in teeth: Siren E, Pedruso E, Haapasalo M. In Vitro disinfection by MTAD, sodium hypochlorite and EDTA of dentin blocks infected by *Enterococcus faecalis*.

- **Summary:**

- Freshly extracted human premolar, canine and maxillary incisor teeth were incubated in 0.5% NaOCl overnight for surface disinfection.
- For infection, the test blocks were stored in each test tube with 5 ml of tryptone soya broth at 37° C with *E. faecalis* strain ATCC 29212 bacteria. The broth was changed at every 2 to 3 days and the incubation continued for 2 weeks.
- The irrigating solutions listed (below) were tested for their antibacterial effect against *E. faecalis*. EDTA was mixed with water into a 17% solution and the pH was adjusted to 7.0 by 1N NaOH. NaOCl was diluted from a 5% stock solution obtained by prescription from the pharmacist. All solutions were freshly prepared before the experiments.
  - a. MTAD (BioPure, 100% solution) 10 min
  - b. NaOCl 1.3% + MTAD (5 + 5 min)
  - c. EDTA 17 % + NaOCl 1.3% (5 + 5 min)
  - d. Sterile water (control) (10 min)
  - e. MTAD 60 min
  - f. NaOCl 1.3% + MTAD (5 + 55 min)
- Bacterial killing was tested by removing sequential 100 micrometer thick layers of dentine with a sterile bur, starting from the main canal towards the periphery of the dentin block. Samples were taken to the depth of 450 micrometers and cultured immediately on culture media and checked for growth at 24 and 48 hours.
- The average number of bacteria per sample in the first zone was  $20,266 \pm 31,390$  (mean  $\pm$  SD) for sterile water. For the BioPure MTAD treatment for 10 minutes (without NaOCl), the average number of cells in the first zone was greatly reduced as compared to the control, the average being less than 1 % of the control values,  $108 \pm 135$  per sample. With *E. faecalis* A197A weak growth was detected in only one sample in the MTAD 10 min group (Group 1) using the tryptone soya broth culture (non-quantitative test). No growth was detected in the samples taken from the other groups.

**Summary of *E. faecalis* culture results in teeth:** Both research groups (Loma Linda (surface disinfection) and the University of Helsinki, Finland (dentin block disinfection)) determined that *E. faecalis* (2 strains) is eliminated in teeth/dentine by following the clinical protocol using BioPure MTAD cleanser with 1.3% NaOCl.

5. University of Oslo, Norway : Antibacterial Studies

Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of *Enterococcus faecalis* by MTAD, chlorhexidine digluconate and a combination of chlorhexidine digluconate and cetrimide in the presence and absence of potential inhibitors.

- **Summary:**

- A study is underway at the University of Oslo in Norway to evaluate the killing of *E. faecalis* with various intra-canal rinses. In their tests, two medicaments are being tested:
  - a. Bio-Pure MTAD Root Canal cleanser (DENTSPLY Tulsa Dental, Tulsa, USA), and
  - b. chlorhexidine digluconate (Sigma Chemicals Co., St. Louis, MO, USA).
- Their ability to kill *E. faecalis* is being tested in the presence of dentin or bovine serum albumin and at various dilutions. Two strains of *E. faecalis* bacteria are being used, to illustrate the variability of the organism:
  - a. strain A197A, isolated from a persistent apical periodontitis, and
  - b. strain VP3-80, isolated from a previously root-filled tooth with chronic apical periodontitis.
- Both strains of *E. faecalis* bacteria were reduced within seconds by both 100% BioPure MTAD and 0.2% chlorhexidine. After 5 minutes of treatment, no viable cells of strain *E. faecalis* A197A were detected. The VP3-80 strain of *E. faecalis* was slightly more resistant to BioPure MTAD and chlorhexidine solutions. Diluting the BioPure MTAD cleanser to 10% of its original concentration reduced the speed of killing, but the bacteria were killed within 20 minutes for one strain and 60 minutes for the other. Because the doxycycline binds to dentin, the drug and its effects may be available to the body for more than the 5-minute treatment. This means that the BioPure MTAD cleanser may continue to kill bacteria after the cleanser liquid is removed from the canal. However, extreme dilution (to 1%) fails to eliminate bacteria during a 1-hour incubation period.
- Another dilution experiment was conducted with inhibitors: dentin & bovine serum albumin (to simulate human collagen). BioPure MTAD and 0.2% chlorhexidine were affected by dentin and bovine serum albumin. Both dilution and the inhibitors delayed the killing of *E. faecalis*. It took up to 24 hours to kill the bacteria with the addition of dentin powder. The bovine serum albumin was less inhibitory and virtually all bacteria were killed in this test with dilute solutions after 1 hour.

6. NAMSA (North American Science Associates): Zone of Inhibition and Minimum Inhibitory Concentration Testing

- **Summary:**

- Zone of inhibition tests were performed by NAMSA with BioPure MTAD solutions. The Kirby-Bauer zone of inhibition test method was used with 8 replicates with *E. faecalis* bacteria. These samples were inoculated at 37°C for 18 to 24 hours and 8 replicates were tested and the results were assessed as inhibitory or non-inhibitory by NAMSA.
- The zone of inhibition tested was performed to determine that inhibition of growth occurred with fresh samples and aged samples. One sample had been aged for 6 months and second had been subjected to higher temperature and humidity (40°C/75% relative humidity for 15 weeks) to simulate aging for 1 year at room temperature.
- Minimum inhibitory concentration testing was also conducted by NAMSA.
- The fresh, the aged and the accelerated testing samples all had inhibitory effects on the growth of *E. faecalis* bacteria. These diameters at NAMSA show inhibition of growth of *E. faecalis* bacteria. The zones of inhibition were roughly the same for all samples.
- In their minimum inhibitory concentration tests, no growth of *E. faecalis* bacteria was observed up to a dilution of 1 in 2048, a higher effective dilution than measured by Loma Linda University. At NAMSA, *E. faecalis* was used with  $1.2 \times 10^5$  colony forming units/ml.

**Independent Testing Summary:** Three studies at independent locations have validated, using standard in vitro models, that *E. faecalis* bacteria is killed by BioPure MTAD root canal cleanser. All 3 sites confirm the killing of *E. faecalis* bacteria. The test results for zone of inhibition and minimum inhibitory concentration are similar in their results at the two sites using this technique. However, the strain of *E. faecalis* bacteria did affect the test results. Two strains were tested and the BioPure MTAD cleanser was effective against both. Aged samples (zone of inhibition tests) to date show no diminution of efficacy of kill. Dentin powder and bovine serum albumin slowed but did not eliminate the killing effect of BioPure MTAD cleanser. As with any medicament, dilution also affected its rate of bacterial killing.

7. Beltz RE, Torabinejad M, Poursmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin J Endodon 2003; 29(5):334-7.

- **Summary :**

- The purpose of this study was to determine the amount of tissue loss after exposing bovine pulp and dentin to MTAD, three concentrations of NaOC, 17% EDTA and isotonic saline.

- Conclusion: The results showed that various concentrations of NaOCl removed organic components of the pulp and dentin effectively. As pulp solubilizers, 5.25% and 2.60% NaOCl were equal (>90%), and 5.25% NaOCl was capable of dissolving virtually the entire organic component of dentin. EDTA was capable of solubilizing inorganic material in dentin and organic material in pulp and most likely in dentin. It dissolved >70% of the dentin and >51% of the pulp. The solubilizing effects of MTAD on pulp and dentin were somewhat to those of EDTA. The major difference between the actions of these solutions was a high binding affinity of doxycycline present in MTAD for the dentin.

### **BIOCOMPATABILITY**

1. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT tetrazolium method. *J Endodon* 2003; 29(10):654-7.

- **Summary:**

- The purpose of this investigation was to test the cytotoxicity of MTAD compared with that of commonly used irrigants and medications.
- Conclusion: Based on the test results, it seems that MTAD is less cytotoxic than eugenol, 3% H<sub>2</sub>O<sub>2</sub>, Ca(OH)<sub>2</sub> paste, 5.25% NaOCl, Peridex and EDTA. The results show MTAD is more cytotoxic than 2.63%, 1.3% and 0.66% NaOCl.
- With the ID<sup>50</sup> at 6750 µg/ml, it seems that the cytotoxicity of MTAD is minimal as evaluated by the MTT-tetrazolium method.

2. Torabinejad, M, Shabahang, S, Bahjri, K. Effect of MTAD on Postoperative Discomfort: A Randomized Clinical Trial. *J Endodon* 2005; 31(3): 171-176.

- **Summary:**

- The purpose of this study was to compare levels of post-operative discomfort after cleaning and shaping of root canals using two protocols for removal of the smear layer.
- Conclusion: No patient developed severe swelling, pain or other side effects necessitating removal from this study. Mild pain decreased gradually in both groups over the seven days.
- A significant change was measured in the mean pain scores over time for both treatment groups (p<0.05). Statistical analysis of the data showed no significant difference in the amount of discomfort experienced in the group that had treatment with EDTA solution or the BioPure MTAD cleanser. No significant differences were detected between the mean post-operative pain scores (six hours to 7 days) between the two groups.
- When the interaction between time and treatment groups was examined, no significant differences were observed between the patterns of change in the pain scores of the two groups (p>0.05).

- No significant differences were observed when pre- and post-operative comparisons of several outcome variables were made.
- BioPure MTAD antibacterial root canal cleanser solution did not cause discomfort in patients who had a final rinse for the removal of the smear layer.

### 3. Mutagenicity Testing – Loma Linda University

- **Summary:**

- The 2<sup>nd</sup> test for the mutagenic potential was conducted using the Ames *Salmonella* /Microsome protocol. In this test, the BioPure MTAD cleanser was compared to 5.25% sodium hypochlorite at 5 doses, using 4 strains of cells. Pyrogen-free water was used as a solvent and as the negative control. The agar test medium and bacteria were incubated at 37°C for 48 hours. S9 rat liver preparation was included to enhance the sensitivity of the test. The plates were examined for revertant colonies of bacteria, indicative of mutagenicity.
- **Conclusion:** The mutagenic potentials of BioPure MTAD and sodium hypochlorite solutions were evaluated using the Ames test. No significant differences were noted between the negative (non-mutagenic) control and the BioPure MTAD or sodium hypochlorite solutions, with or without S9 rat liver cell microsomal activation. Therefore, the BioPure MTAD cleaner and sodium hypochlorite were not mutagenic.

### 4. Machnick TK, Torabinejad M, Munoz CA, Shabahang S. Effect of MTAD on flexural strength and modulus of elasticity of dentin. J Endodon, 2003; 29 (11):747-50.

- **Summary**

- The purpose of this study was to evaluate the effect of MTAD on the flexural strength and modulus of dentin.
- **Conclusion:** Based on the study results it seems that using the clinical protocol for MTAD causes no adverse effects on the physical properties of exposed dentin. The results of this in vitro study suggest that MTAD possesses most of the positive qualities of an ideal root canal irrigant.

## **BOND STRENGTH**

### 1. Machnick TK, Torabinejad M, Munoz CA, Shabahang, S. Effect of MTAD on the bond strength to enamel and dentin. J Endodon 2003; 29(12):818-21.

- **Summary:**

- This study was conducted to compare the effect of MTAD ((a mixture of tetracycline isomer, an acid (citric acid), and a detergent (Tween 80)) and a phosphoric acid on the bond strength to enamel using a conventional OptiBond Solo Plus dentin adhesive system.
- **Conclusion:** According to these findings, it appears that teeth endodontically treated with MTAD protocol for clinical use (20

minutes 1.3% NaOCl/ 5 minutes with MTAD) may not need additional conditioning before the application of the dental adhesive.

### **CORONAL LEAKAGE**

1. Park DS, Torabinejad M, Shabahang S. J Endod. 2004; 30(12):890-2. The Effect of MTAD on the coronal leakage of obturated root canals.

- **Summary**

- The effect of the BioPure MTAD antibacterial root canal cleanser was compared to that of other materials for preventing coronal leakage. The India ink penetration method was used with fifty non-carious, extracted, human, single-rooted teeth with closed apices.
  - a. 5.25% NaOCl; no sealer, obturation with gutta-percha (positive control),
  - b. 5.25% NaOCl; AH plus sealer, obturation with gutta-percha, and sticky wax (negative control).
  - c. 5.25% NaOCl; AH plus sealer, obturation with gutta-percha,
  - d. 5.25% NaOCl, 5 ml of 17% EDTA for 5 minutes, 5.25% NaOCl; AH plus sealer, obturation with gutta-percha and, or
  - e. 1.3% NaOCl, 5 ml of BioPure MTAD for 5 minutes; AH plus sealer, obturation with gutta percha.
- In the first group (positive control), the dye penetrated the entire length of each tooth's canal. No dye penetration was measured for the negative control- group 2. The "NaOCl only" group (#3) experienced more microleakage than did the group with BioPure MTAD cleanser and NaOCl (#5). Numerically, the EDTA group (#4) had more microleakage than the BioPure MTAD group (#5), but the difference was not statistically significant.

### **DISCOLORATION**

1. Tetracycline binds to calcium in the hydroxylapatite crystals during the development stage of hardening or calcifying of teeth. This chelation of the tetracycline leads to permanent tooth discoloration in the permanent teeth as they develop, or in deciduous teeth. To avoid discoloration, tetracycline products, including BioPure MTAD cleanser, should NOT be taken by young children with developing teeth ( $\leq 8$  years old). Similarly, pregnant or nursing mothers should NOT take tetracycline-containing drugs because of the transmission of the drug to the child. Transmission of the drug may affect the teeth during development (from the fourth month in the uterus). Since the total dosage is more important the total period of administration, this contraindication should be abided by strictly.

With adults, the teeth are fully developed so that the discoloration will not occur. (HS. The mechanism of tetracycline staining in primary and permanent teeth. J Dent Child 1967; 34(6):478-87).

Chlorhexidine containing products do extrinsically stain teeth, but BioPure MTAD cleanser does not contain chlorhexidine.

### **SMEAR LAYER REMOVAL**

1. Torabinejad M, Handysides R, Khademi, AA, Bakland LK. Clinical implications of the smear layer in endodontics. Oral Surg 2002; 94:658-66.

- **Summary:**

- The purpose of this article was to review current evidence regarding clinical implications of the smear layer in endodontics.

2. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S. A new solution for the removal of the smear layer. J Endodon 2003; 29:170-5.

- **Summary:**

- The purpose of this study was to evaluate the effectiveness of an irrigant solution, a mixture of a tetracycline isomer, an acid, and a detergent (MTAD), to disinfect the dentin, remove the smear layer. Open the dentinal tubules and allow the antibacterial agents to penetrate the entire root canal system.
- Conclusion: Based on the results of this study, it seems that MTAD is an effective solution for the removal of the smear layer when used as a final rinse. It does not significantly change the structure of the dentinal tubules when used in conjunction with NaOCl as a root canal irrigant.
- The results demonstrated that MTAD is also less destructive to the tooth structure compared with EDTA when used as a final irrigant. Close examination of the appearance of the dentinal tubules showed higher amounts of erosion with EDTA. This study also showed no significant difference in the ability of distilled water and NaOCl to remove the smear layer from the surfaces of instrumented canals because both irrigants were ineffective.

3. University of North Carolina: Smear Layer Removal/SEM's

- **Summary:**

- Partial results are available from the University of North Carolina. The smear layer remained when either the distilled water or the 1.5% NaOCl rinse was used. BioPure MTAD and EDTA solutions were equally effective in smear layer removal, producing a clean dentin surface. No comparisons using the rating system for the areas of the root canals are yet available. However, the efficacy of BioPure MTAD cleanser in removing the smear layer is clear. Both EDTA solution and BioPure MTAD cleanser were effective in removing the smear layer.

4. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *J Endodon* 2003(4); 29:233-9.

- **Summary:**

- The purpose of this study was to investigate the effectiveness of various concentrations of sodium hypochlorite (NaOCl) as an intracanal irrigant before the use of MTAD (a mixture of tetracycline isomer, an acid and a detergent) as a final rinse to remove the smear layer.
- **Conclusion:** The results show that although MTAD removes most of the smear layer when used as an intracanal irrigant, some remnants of the organic component of the smear layer remain scattered on the surface of the root canal walls. The effectiveness of MTAD to completely remove the smear layer is enhanced when low concentrations of NaOCl are used as an intracanal irrigant before the use of MTAD as a final rinse. The regimen does not seem to significantly change the structure of dentinal tubules.